Rainforest refugia and evolution in Australia's Wet Tropics

Chris Schneider and Craig Moritz[*]

Cooperative Research Centre for Tropical Rainforest Ecology and Management, Department of Zoology, University of Queensland, St Lucia, QLD 4072, Australia

Comparison of mitochondrial DNA variation among populations of three endemic lizard species in Australia's Wet Tropics rainforest reveals clear evidence of population-level response to Pleistocene rainforest contraction and subsequent expansion. This pattern is repeated in two separate rainforest regions (natural replicates), between which populations have been separated for several million years. Despite evidence for isolation in rainforest refugia, historically reduced effective population size, and recent range expansion, little or no detectable phenotypic evolution has occurred among populations, even those isolated for several million years. These observations suggest that long-term isolation *per se*, even when combined with major reductions in population size and opportunities for drift and founder effects, is unlikely to be the primary process driving phenotypic divergence and speciation of rainforest vertebrates.

Keywords: Pleistocene refugia; speciation; tropical rainforest

1. INTRODUCTION

The role of Pleistocene rainforest refugia in phenotypic divergence among populations and speciation is a persistent question in evolutionary biology and ecology. For nearly 30 years the Pleistocene refuge model of speciation (Haffer 1969; Vanzolini & Williams 1970) has been a dominant paradigm for vertebrate diversification in the tropics (see Prance 1982; Haffer 1997, for reviews), but despite several critical evaluations (see, for example, Endler 1982; Bush 1994; Colinvaux *et al.* 1996) the large spatial scale and inaccessibility of most tropical regions, coupled with poor palaeopalynological records and poor taxonomic resolution for most vertebrate groups, have made direct tests of this hypothesis difficult.

The critical issues in examining the Pleistocene refuge model of diversification are (i) whether there is evidence that rainforests were fragmented into isolated refugia during Pleistocene glacial periods, (ii) whether vertebrate populations in the rainforest were fragmented as a result, and (iii) whether isolation in rainforest refuges resulted in significant phenotypic divergence or speciation. Palynological evidence of rainforest contraction during Pleistocene ice ages is sparse for most tropical regions and existing data from Amazonia (one of the better-sampled regions) are ambiguous regarding the extent and nature of changes in rainforest distribution and composition during Pleistocene ice ages (Colinvaux 1987, 1997; Colinvaux *et al.* 1996; Hooghiemstra 1997; Van der Hammen & Absy 1994). In sharp contrast, pollen cores from the Wet Tropics rainforest of Australia reveal a clear history of severe rainforest contraction during Pleistocene glacial

periods (Kershaw 1983, 1993, 1994). Additional evidence comes from analyses of fossil charcoal, which indicate that even likely ice-age refuges were deeply dissected by fire-prone vegetation (Hopkins *et al.* 1993). These data are consistent with palaeoclimate models, which predict that suitable temperature and moisture regimes would have been fragmented and disjunct throughout the region (Nix 1991). In total, this large body of evidence unambiguously demonstrates that rainforests in the Wet Tropics were severely fragmented and reduced in area during Pleistocene glacials. Subsequent to the last major glacial, there is evidence that rainforest expanded during a more favourable climatic period *ca.* 7500 years before present (Nix 1991; Hopkins *et al.* 1996). This expansion united currently disjunct blocks of rainforest and provided the opportunity for colonization of areas where endemic vertebrates were absent or recently extinct (see Winter 1997; Schneider *et al.* 1998). Previous analyses of mtDNA variation in birds, frogs and lizards (Joseph *et al.* 1995; Schneider *et al.* 1998) reveals evidence of recent colonization of some areas that is consistent with this scenario.

Given that rainforests in the Australian Wet Tropics were severely fragmented during Pleistocene glacials, we here examine patterns of mtDNA variation in three endemic lizard species that have low vagility and therefore should have been strongly affected by Pleistocene rainforest contraction. Previous studies have demonstrated that speciation events in nearly all endemic vertebrates in the Wet Tropics occured before the Pleistocene (Joseph & Moritz 1993; Joseph *et al.* 1995; Moritz *et al.* 1997; Schneider *et al.* 1998). This conclusion holds for the species examined here (whose closest relatives occur outside the Wet Tropics; C. J. Schneider and C. Moritz, unpublished data) and also for microhylid frogs (*Cophixalus*; C. Hoskin

Proc. R. Soc. Lond. B (1999) **266**, 191–196
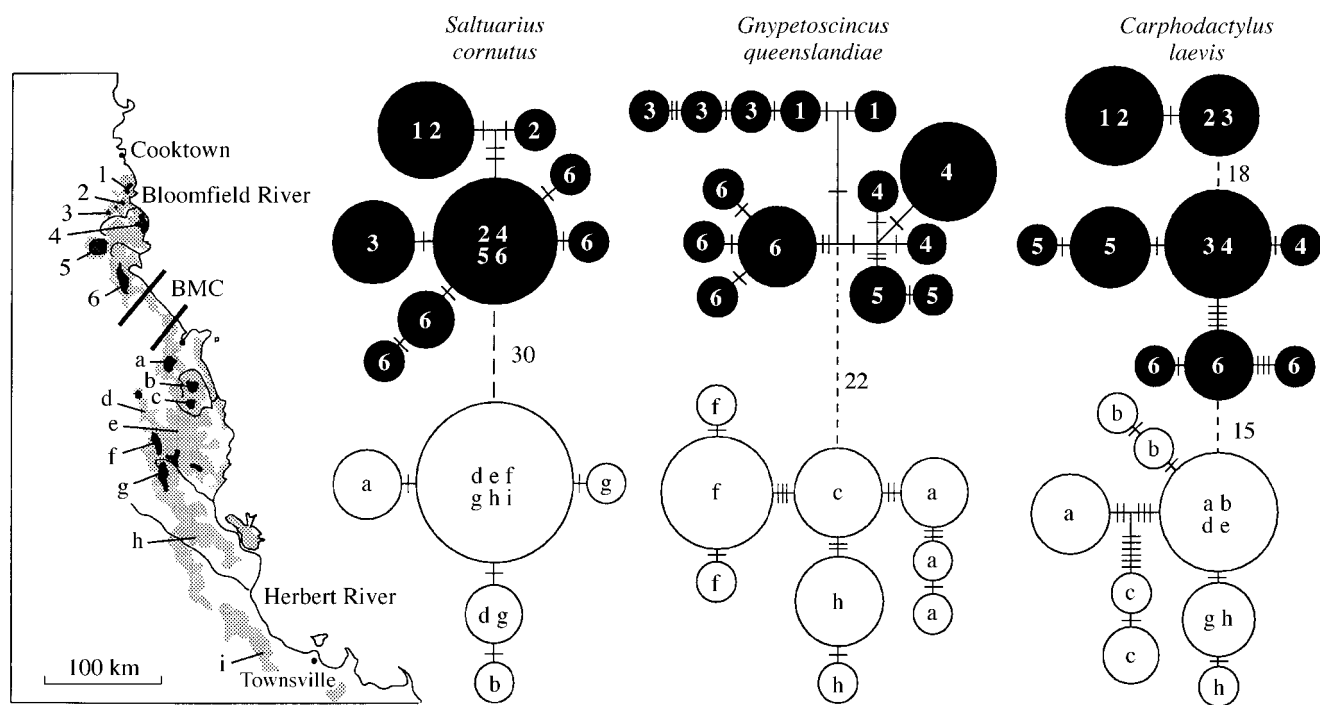*Received* 1 October 1998   *Accepted* 8 October 1998

191

Figure 1. The Wet Tropics World Heritage area of north-east Queensland showing sampling localities, current distribution of rainforest (light grey), suggested Pleistocene refugial areas (dark grey; from Webb & Tracey 1981), and minimum-length unrooted trees of mtDNA haplotypes for each species. The area of the circles on haplotype trees is scaled to reflect the frequency of haplotypes, with the smallest circle equal to one and the largest circle equal to 16. Trees were generated by using PAUP*4.0d63 (Swofford 1998). Hash marks denote single base substitutions and dashed lines indicate uncertain connections between divergent mtDNA groups. Values next to dashed lines are the minimum numbers of substitutions between divergent groups.

and C. Moritz, unpublished data), the only vertebrate genus that has radiated within the Wet Tropics. In this paper we do not attempt to address the mechanisms of ancient speciation in these taxa; rather, we address the question of whether Pleistocene rainforest contraction resulted in fragmentation of vertebrate populations, and whether this historic isolation resulted in phenotypic divergence between isolates.

We first determined whether the lizard species examined here were affected by Pleistocene rainforest contraction by examining mtDNA sequence variation in the light of the following predictions. Species that experienced severe bottlenecks in response to Pleistocene rainforest contraction should show evidence of historically small effective population size as indicated by low overall nucleotide diversity (Birky *et al.* 1983, 1989). Subsequent expansion should be detectable by the presence of broadly distributed ancestral mtDNA alleles (internal alleles in unrooted trees; Templeton *et al.* 1995), a star-like phylogeny of alleles with a few, high-frequency ancestral alleles and numerous low-frequency alleles separated by one or two mutational steps from ancestral alleles (Slatkin & Hudson 1991) and, as a corollary, a Poisson mismatch distribution of pairwise differences among individuals (Rogers & Harpending 1992) and low levels of geographic population structure as measured by Φ_{st} (Excoffier *et al.* 1992). By contrast, species that maintained multiple refugial populations should show strong phylogeographic structure, with exclusive groups of mtDNA haplotypes in areas that served as refuges, high Φ_{st} values (assuming limited gene flow among populations), high

nucleotide diversity overall, and a multimodal mismatch distribution.

2. METHODS

We examined mtDNA sequence variation in three widespread, endemic lizard species confined to mesothermal rainforest in the Wet Tropics (Nix & Switzer 1991; Williams *et al.* 1996). We chose lizards for their limited dispersal ability and ease of collection but our results extend to other vertebrates as well (see Joseph *et al.* 1995; Moritz *et al.* 1997; Schneider *et al.* 1998). The three species examined here are the prickly skink (*Gnypetoscincus queenslandiae*, $n=50$) with sampling additional to that of Joseph *et al.* (1995); the leaf-tailed gecko (*Saltuarius cornutus*, $n=49$); and the chameleon gecko (*Carphodactylus laevis*, $n=48$). All of these species are endemic to the Wet Tropics of Australia, are restricted to deep rainforest habitat, and have similar ranges except that *S. cornutus* extends to the southern limit of the Wet Tropics, whereas *G. queenslandiae* and *C. laevis* have their southern limit at the Herbert River (figure 1). Each species was examined over its full geographic range, including the majority of putative refugia (figure 1), with sample sizes per locality varying from two to eight.

We identified mitochondrial haplotypes by amplifying and directly sequencing 357 bp of the mitochondrial cytochrome *b* gene (cyt *b*) from *G. queenslandiae* by using primers described in Joseph *et al.* (1995), and 372 bp from the same region of cyt *b* in *C. laevis* and *S. cornutus* by using primers Ph1 (5′GACCCCAA-TACGAAAAACYCACCC 3′) and Cyt *b*2 (Kocher *et al.* 1989) with standard PCR and sequencing protocols. All sequences have been submitted to EMBL (accession no. AF109473–619).

Table 1. *Summary of mtDNA variation within and between regions in the Wet Tropics*

(π (nucleotide diversity) and Φ_{st} were calculated by using Arlequin (Schneider *et al.* 1992) for uncorrected pairwise differences among sequences. Values for *C. laevis* north of the Black Mountain Corridor (BMC) do not include the divergent mtDNA group north of the Bloomfield River. Divergence (d_{xy}) across the BMC and maximum sampling variance were estimated using the method of Takahata & Tajima (1991) on Kimura two-parameter distances among sequences.)

		S. cornutus	*C. laevis*	*G. queenslandiae*
π	north of BMC	0.005 ± 0.0033	0.008 ± 0.0046	0.009 ± 0.0054
	south of BMC	0.002 ± 0.0016	0.010 ± 0.0058	0.009 ± 0.0054
	total	0.045 ± 0.0225	0.039 ± 0.0191	0.037 ± 0.0190
Φ_{st}	north of BMC	0.247	0.828	0.830
	south of BMC	0.401	0.801	0.919
	total	0.980	0.956	0.971
d_{xy}	across BMC	0.091 ± 0.0167	0.048 ± 0.0124	0.066 ± 0.0143

Estimates of nucleotide diversity and population structure were obtained by means of Arlequin (Schneider *et al.* 1996) with Φ_{st} estimated from the observed number of pairwise differences between sequences.

Phenotypic variation was assessed from museum specimens of adult *S. cornutus* ($n=35$) and *C. laevis* ($n=24$) by C.J.S. and adult *G. queenslandiae* ($n=80$) by M. Cunningham. Ecomorphological characters (limb length, body size, head length and gape width) were measured with dial calipers to the nearest 0.1 mm except for body size (snout–vent length), which was measured with a ruler to the nearest 1.0 mm. Scale characters examined varied among species but included characters typically used for species diagnoses within each taxonomic group. Morphological characters are all continuously and normally distributed and were analysed with multivariate analyses of variance (MANOVA) among regions and populations with univariate *F*-tests for individual character differences.

3. RESULTS

(a) *Genetic evidence for Pleistocene (and earlier) vicariance*

Previous phylogeographic analyses of mtDNA variation in birds, lizards (including the species examined here) and frogs (Joseph *et al.* 1995; Schneider *et al.* 1998) identified a large genetic break in all species centred on a region known variously as the Black Mountain Barrier or Black Mountain Corridor (BMC in figure 1). The magnitude of the genetic distances across the BMC suggests pre-Pleistocene isolation (Schneider *et al.* 1998) and therefore the regions on either side of the BMC provide two natural replicates with which to examine the effects Pleistocene rainforest contraction on patterns of mtDNA variation and phenotypic evolution.

Patterns of mtDNA sequence variation within each region are consistent with expectations of Pleistocene fragmentation, isolation, and reduction. These patterns are remarkably consistent within species on both sides of the BMC, but differ substantially among species. It is notable that *C. laevis* and *G. queenslandiae* contain relatively high nucleotide diversity within regions and concomitantly lower divergence between regions (table 1). We interpret this difference as a result of larger long-term effective population size in *C. laevis* and *G. queenslandiae* relative to *S. cornutus*. North of the BMC, *S. cornutus* appears to have

been reduced to two populations, probably one on either side of the Bloomfield River (figure 1), and the depth of divergence among these populations (1.1% observed sequence difference) is consistent with expectations of Pleistocene divergence. The presence of two refugial populations is reflected in the bimodal mismatch distribution of substitutions among individuals (figure 2*a*). In addition, *S. cornutus* shows little population structure across the region relative to the other species ($\Phi_{st}=0.247$) and the presence of a widespread, common, ancestral haplotype (figure 1) suggests recent expansion from one of the refugial populations. In contrast, both *G. queenslandiae* and *C. laevis* show evidence of having maintained multiple refugial populations in each of the major rainforest blocks north of the BMC. Both species show high nucleotide diversity and strong phylogeographic structure with unique groups of mtDNA (excluding the secondary contact in *C. laevis* at locality 3) confined to each of the major upland rainforest blocks (figure 1). Levels of sequence divergence among blocks are broadly consistent with expectations from Pleistocene divergence except for the high divergence in *C. laevis* across the Bloomfield River, which reflects a much older separation. The strong geographic structure of mtDNA variation among regions north of the BMC is reflected by high Φ_{st} values (table 1), and multimodal mismatch distributions (figure 2*c,e*) for both *C. laevis* and *G. queenslandiae*.

South of the BMC, *C. laevis* and *G. queenslandiae* again show strong local phylogeographic structure (figure 1), high Φ_{st} values, high nucleotide diversity (table 1) and multimodal mismatch distributions (figure 2*d,f*) consistent with multiple refugial populations. Interestingly, the distribution of divergent haplotype groups is concordant with predicted refuges (Webb & Tracey 1981) at locations a, c, and f in figure 1. By contrast, and as in the north, *S. cornutus* shows low overall nucleotide diversity and low Φ_{st} values (table 1). The presence of a common, widespread, ancestral haplotype, a star-like phylogeny of alleles (figure 1) and the concomitant unimodal mismatch distribution (figure 2*b*) are consistent with recent population expansion from a single refugial source. Recent range expansion is also indicated by the sharing of internal alleles over broad geographic distances and across current barriers to dispersal (e.g. between localities h and i in figure 1).
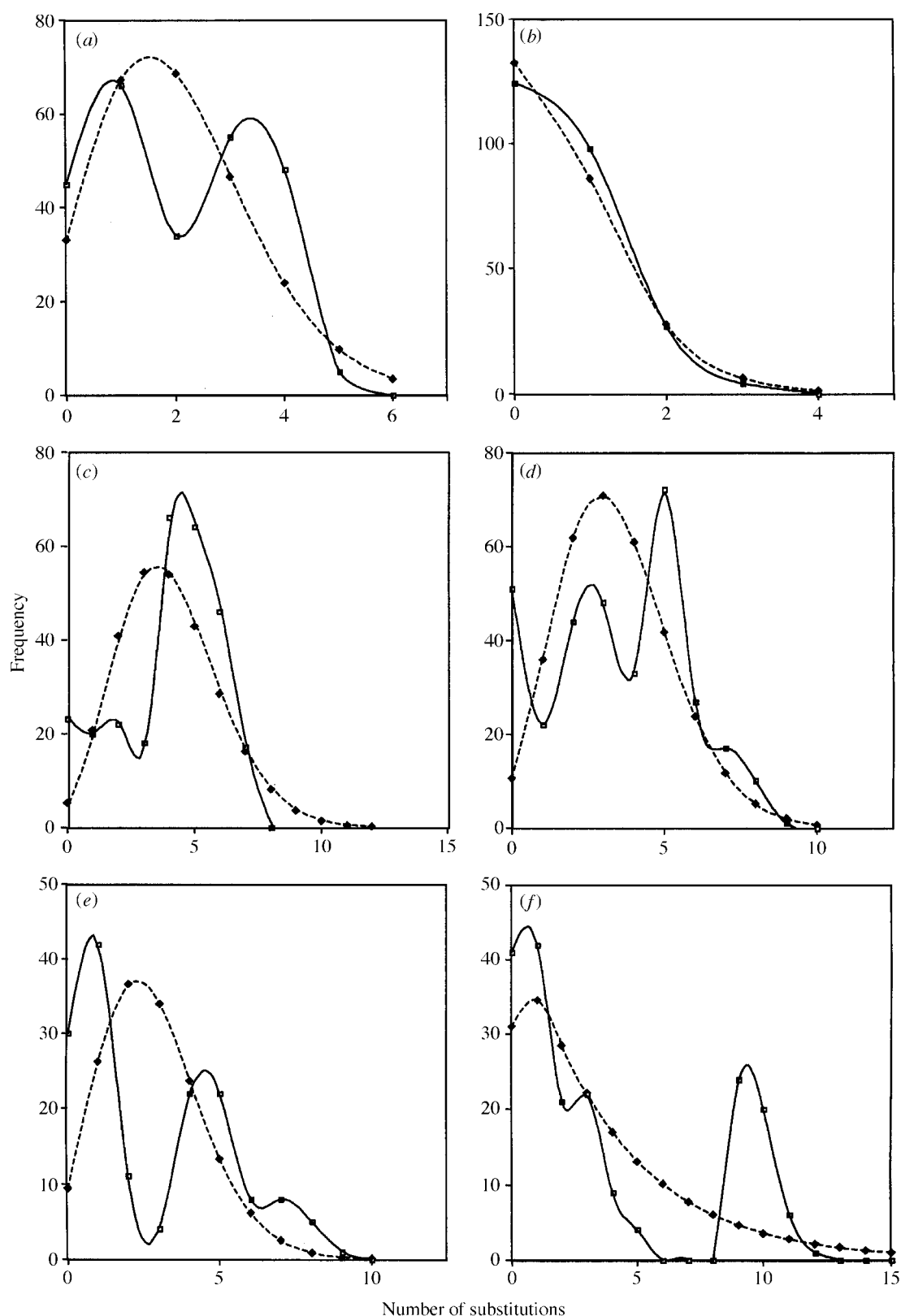
Figure 2. Observed distribution (solid line) of pairwise differences among individuals and Poisson distribution expected from an expanding population (dashed line). We tested the fit of the observed data to the Poisson expectation with a single-classification G-test. Listed p-values are the probability of the observed distribution fitting the Poisson expectation. (*a*) *Saltuarius cornutus* north of the BMC. The bimodal distribution does not fit a Poisson distribution (probability of fit to Poisson, $p < 0.01$); however, if the relatively divergent haplotypes from north of the Bloomfield River are excluded, the observed distribution fits the Poisson expectation ($0.50 > p > 0.10$). (*b*) *Saltuarius cornutus* south of the BMC. The observed distribution fits a Poisson distribution and is consistent with recent expansion from a single refugial population ($p = 0.50$). (*c*) *Gnypetoscincus queenslandiae* north of the BMC and (*d*) *Gnypetoscincus queenslandiae* south of the BMC. The multimodal mismatch distribution does not fit Poisson expectations of expansion from a single source ($p < 0.001$) and suggests multiple refugial populations. Sample sizes and amount of genetic (*Cont.*)

Table 2. *Probability that populations isolated on either side of the BMC differ in five ecomorphological characters*

(Values are derived from a MANOVA comparing adults on either side of the BMC. There is no significant variation in scalation or other external morphology among populations within regions on either side of the BMC (data not shown).)

	S. cornutus	*C. laevis*	*G. queenslandiae*
snout–vent	0.253	0.645	0.104
hind limb	0.553	0.937	0.134
front limb	0.493	0.907	0.999
gape width	0.412	0.995	0.119
head length	0.466	0.815	0.657
Wilk's λ	0.632	0.383	0.521

(b) *Phenotypic variation*

The patterns of mtDNA diversity in both regions on either side of the BMC are largely consistent with expected patterns of variation resulting from fragmentation, reduction, and subsequent expansion in response to Quaternary climate change. Populations of *S. cornutus* in particular appear to have been severely restricted, with perhaps a single refugial population on either side of the BMC. However, these large population fluctuations, even when combined with several million years of isolation across the BMC, had little effect on phenotypic divergence. Populations of *S. cornutus* and *C. laevis* on either side of the BMC (including the genetically divergent populations of *C. laevis* north of the Bloomfield River) are indistinguishable in univariate or multivariate analyses (table 2; univariate p-values for individual characters all greater than 0.10 for *S. cornutus* and greater than 0.60 for *C. laevis*). Similarly, populations of *G. queenslandiae* do not differ in the five ecomorphological characters across the BMC (table 2; univariate p-values all greater than 0.10) and most scale characters, but they do differ in a minor scale character (mean number of paravertebral scales: north 35.72, south 40.24, $p < 0.01$). That these species show little or no morphological divergence, even among populations isolated for several million years, suggests that isolation, even when combined with severe population reductions, has been insufficient to generate phenotypic divergence.

4. DISCUSSION

Palynological and other data unambiguously demonstrate that rainforest in the Wet Tropics underwent dramatic changes in distribution, extent and composition in response to Quaternary climate changes. Mitochondrial DNA sequence variation in the three endemic rainforest species examined here is consistent with fragmentation, isolation and reduction in response to Pleistocene rainforest contraction. In addition, there are deeper vicariant events that isolated populations of each of these species on either side of the BMC and, in the case of *C. laevis*, on either side of the Bloomfield River. This history of contraction, isolation and subsequent expansion provides ample opportunity for phenotypic divergence through drift and founder effects, yet, even in the most extreme case of long-term isolation (across the BMC), we do not see evidence of phenotypic divergence. The morphological stasis exhibited by the three lizard species is striking, but is not unique among vertebrates, as indicated by the lack of recognized taxonomic differentiation across the BMC in well-known groups such as birds and mammals. The sole exception is two species of ringtail possum (*Pseudocheirus herbertensis* and *P. cinereus*), which may be a case of recent chromosomal divergence (Moritz *et al.* 1997).

We suspect that the lack of phenotypic divergence results from relatively stable selective regimes even in small rainforest refuges where species were able to persist through Pleistocene glacial periods. Rather than resulting in adaptive divergence, isolation in rainforest refuges appears to have resulted in localized extinction of some species, a process that is reflected in patterns of species diversity as well as mtDNA variation (Williams & Pearson 1997). Regardless of the mechanisms maintaining morphological similarity, the fact remains that long-term isolation of, at times, small effective populations and subsequent range expansion has not resulted in phenotypic divergence. This finding directs our search for processes producing diversity in tropical rainforests away from drift and founder effects in small isolates (Hewitt 1996; see, for example, Roy 1997; Fjeldså & Lovett 1997), and towards differences in selective regimes that may result in phenotypic divergence and speciation whatever the geographic arrangement of populations. In this context the role of divergent selection across environmental gradients (Endler 1982; Smith *et al.* 1997) or among isolates in topographically complex areas warrants further investigation.

REFERENCES

Birky, C. J., Maruyama, T. & Fuerst, P. 1983 An approach to population and evolutionary genetic theory for genes in mitochondria and chloroplasts, and some results. *Genetics* **103**, 513–527.

Birky, C. J., Fuerst, P. & Maruyama, T. 1989 Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. *Genetics* **121**, 613–627.

Figure 2. (*Cont.*) variation are inadequate to test for expansion from each putative refugial population. (*e*) *Carphodactylus laevis* north of the BMC (excluding the divergent haplotypes found north of the Bloomfield River). Again, the multimodal distribution is inconsistent with a Poisson distribution resulting from expansion from a single refugial source ($p < 0.001$) and suggest multiple refugial populations. (*f*) *Carphodactylus laevis* south of the BMC. The multimodal distribution does not fit the expectation of expansion from a single source ($p < 0.001$) and again suggests multiple refugial populations.

Bush, M. B. 1994 Amazonian speciation: a necessarily complex model. *J. Biogeogr.* **21**, 5–17.

Colinvaux, P. A. 1987 Amazon diversity in light of the paleoecological record. *Q. Sci. Rev.* **6**, 93–114.

Colinvaux, P. A. 1997 *The Ice-Age Amazon and the problem of diversity.* Rotterdam: Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

Colinvaux, P. A., de Oliveira, P. E., Moreno, J. E., Miller, M. C. & Bush, M. B. 1996 A long pollen record from lowland Amazonia: forest and cooling in glacial times. *Science* **274**, 85–88.

Endler, J. 1982 Pleistocene forest refuges: fact or fancy? In *Biological diversification in the tropics* (ed. G. T. Prance), pp. 641–657. New York: Columbia University Press.

Excoffier, L., Smouse, P. E. & Quattro, J. M. 1992 Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Fjeldså, J. & Lovett, J. C. 1997 Geographical patterns of old and young species in African forest biota: the significance of specific montane areas as evolutionary centres. *Biodiv. Conserv.* **6**, 325–346.

Haffer, J. 1969 Speciation in amazonian forest birds. *Science* **165**, 131–137.

Haffer, J. 1997 Alternative models of speciation in Amazonia: an overview. *Biodiv. Conserv.* **6**, 451–476.

Hewitt, G. M. 1996 Some genetic consequences of ice ages, and their role in divergence and speciation. *Biol. J. Linn. Soc.* **58**, 247–276.

Hooghiemstra, H. 1997. *Tropical rain forest versus savanna: two sides of a precious medal*? Rotterdam: Nederlandse Organisatie voor Wetenschappelijk Onderzoek.

Hopkins, M. S., Ash, J., Graham, A. W., Head, J. & Hewett, R. K. 1993 Charcoal evidence of the spatial extent of the *Eucalyptus* woodland expansions and rainforest contractions in north Queensland during the late Pleistocene. *J. Biogeogr.* **20**, 59–74.

Hopkins, M. S., Head, J., Ash, J. E., Hewett, R. K. & Graham, A. W. 1996 Evidence of a Holocene and continuing recent expansion of lowland rain forest in humid, tropical North Queensland. *J. Biogeogr.* **23**, 737–745.

Joseph, L. & Moritz, C. 1993 Phylogeny and historical aspects of the ecology of eastern Australian scrubwrens: evidence from mitochondrial DNA. *Molec. Ecol.* **2**, 161–170.

Joseph, L., Moritz, C. & Hugall, A. 1995 Molecular support for vicariance as a source of diversity in rainforests. *Proc. R. Soc. Lond.* B **260**, 177–182.

Kershaw, A. P. 1983 A Holocene pollen diagram from Lynch's crater, north-eastern Queensland, Australia. *New Phytol.* **94**, 669–682.

Kershaw, A. P. 1993 A quaternary vegetation history of north-eastern Queensland from pollen analysis of ODP site 820. *Proc. Ocean Drill. Progr. Scient. Results* **133**, 107–114.

Kershaw, A. P. 1994 Pleistocene vegetation of the humid tropics of northeastern Queensland, Australia. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **109**, 399–412.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X. & Wilson, A. C. 1989 Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natn. Acad. Sci. USA* **86**, 6196–6200.

Moritz, C., Joseph, L., Cunningham, C. & Schneider, C. J. 1997 Molecular perspectives on historical fragmentation of Australian tropical and subtropical rainforest: implications for conservation. In *Tropical forest remnants* (ed. W. Laurance & R. Bierregard), pp. 442–454. University of Chicago Press.

Nix, H. A. 1991 Biogeography: patterns and process. In *Rainforest animals. Atlas of vertebrates endemic to Australia's wet tropics* (ed. H. A. Nix & M. Switzer), pp. 11–39. Canberra: Australian Nature Conservation Agency.

Nix, H. A. & Switzer, M. (eds) 1991 *Rainforest animals. Atlas of vertebrates endemic to Australia's wet tropics*. Canberra: Australian Nature Conservation Agency.

Prance, G. T. (ed.) 1982 *Biological diversification in the tropics*. New York: Columbia University Press.

Rogers, A. R. & Harpending, H. 1992 Population growth makes waves in the distribution of pairwise genetic differences. *Molec. Biol. Evol.* **9**, 552–569.

Roy, M. S. 1997 Recent diversification in African greenbuls (Pycnonotidae: *Andropadus*) supports a montane speciation model. *Proc. R. Soc. Lond.* B **264**, 1337–1344.

Schneider, S., Kueffer, J.-M., Roessli, D. & Excoffier, L. 1996 *Arlequin: an exploratory population genetics software environment*. Available from http://anthropologie.unige.ch/arlequin.

Schneider, C. J., Cunningham, M. & Moritz, C. 1998 Comparative phylogeography and the history of vertebrates endemic to the Wet Tropics rainforest of Australia. *Molec. Ecol.* **7**, 487–498.

Slatkin, M. & Hudson, R. R. 1991 Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. *Genetics* **129**, 555–562.

Smith, T. B., Wayne, R. K., Girman, D. J. & Bruford, M. W. 1997 A role for ecotones in generating rainforest biodiversity. *Science* **276**, 1855–1857.

Swofford, D. L. 1998 PAUP* (phylogenetic analysis using parsimony) test version 4.0d63. Sunderland, MA: Sinauer Associates.

Takahata, N. & Tajima, F. 1991 Sampling errors in phylogeny. *Molec. Biol. Evol.* **8**, 494–502.

Templeton, A., Routman, E. & Phillips, C. 1995 Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the Tiger Salamander, *Ambystoma tigrinum*. *Genetics* **140**, 767–782.

Van der Hammen, T. & Absy, M. L. 1994 Amazonia during the Last Glacial. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **109**, 247–261.

Vanzolini, P. E. & Williams, E. E. 1970 South American anoles: the geographic differentiation and evolution of the *Anolis chrysolepis* species group (Sauria: Iguanidae). *Arq. Zool. São Paulo* **19**, 1–298.

Webb, L. & Tracey, J. 1981 Australian rainforests: pattern and change. In *Ecological biogeography of Australia* (ed. J. A. Keast), pp. 605–694. The Hague: W. Junk.

Williams, S. E. & Pearson, R. G. 1997 Historical rainforest contractions, localized extinctions and patterns of vertebrate endemism in the rainforests of Australia's wet tropics. *Proc. R. Soc. Lond.* B **264**, 709–716.

Williams, S. E., Pearson, R. G. & Walsh, P. J. 1996 Distributions and biodiversity of the terrestrial vertebrates of Australia's wet tropics. *Pacif. Conserv. Biol.* **2**, 327–362.

Winter, J. W. 1997 Responses of non-volant mammals to late Quaternary climatic changes in the Wet Tropics region of northeastern Australia. *Wildl. Res.* **24**, 493–511.